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SO VET.MED. (76, NO.8, 1185-86, 1981).*

SO Vet.Med. (82, No. 6, 646-50, 1987) 2 Tab. 23 Ref. *

SO VETERINARY PARASITOLOGY, (MAY 1993) Vol. 47, No. 3-4, pp. 225-233.

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SO Revista Brasileira de Parasitologia Veterinaria, (1995) Vol. 4, No. 1, pp.* 15-19.

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Effect of disinfectants on survival of cryptosporidium oocysts

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Veterinary Record (1982) **111**, 414-415

The survival of cryptosporidium oocysts after 18 hours' incubation in seven disinfectants was examined by light microscopy and oral inoculation of mice. Only formol saline and ammonia were effective in destroying the viability of the oocysts.

CRYPTOSPORIDIUM is a protozoan parasite (Family: Cryptosporidiidae) which completes its life cycle on the mucosal surface of the bowel of vertebrates (Levine 1973). Cryptosporidium was shown to be associated with diarrhoea in calves (Pohlenz and others 1978, Tzipori and others 1980c) and in other ruminants (Tzipori and others 1981a, Angus and others 1982) as well as in man (Nime and others 1976, Tzipori and others 1980b). The infection appears to be prevalent in animal populations (Tzipori and Campbell 1981b) and the parasite was shown to lack host specificity and therefore is potentially a zoonosis (Tzipori and others 1980a).

The life cycle of cryptosporidium appears to resemble that of other enteric coccidia and infections become established following ingestion of oocysts (Moon and Bemrick 1981). The duration of oocyst survival in the environment is unknown, as is the susceptibility of oocysts to the action of disinfectants. It is therefore difficult to give advice on disinfecting contaminated areas or appropriate precautionary measures to prevent the spread of infection.

Preliminary results on the effect of disinfectants used routinely in the veterinary laboratories (Ministry of Agriculture, Fisheries and Food 1978) for these purposes and their effect on the survival of cryptosporidium oocysts are reported below.

Materials and methods

Cryptosporidia obtained from the gut contents of an experimentally infected calf (Tzipori and others 1980c) were given to newborn specific pathogen free Swiss white mice and wistar rats (Tzipori and others 1980a). Homogenate of whole intestine (20 per cent v/v in phosphate buffered saline) from the infected rats was prepared as a source of cryptosporidium oocysts for these experiments. Seven disinfectants diluted in phosphate buffered saline were mixed with an equal volume of intestinal homogenate. Some of the disinfectants were used at different concentrations. The normal recommended range of concentration (Ministry of Agriculture, Fisheries and Food 1978) is listed in Table 1.

After incubation periods of 10 seconds, two hours and 18 hours, smears of treated homogenates were dried rapidly in a stream of warm air and stained with Giemsa. The 10 second and two hour incubations were at room temperature and the 18 hour incubation at 4°C. To assess the effects of the solutions on oocyst morphology and staining affinity the number of oocysts observed during a two minute period of microscopical examination of each slide was recorded (Table 2).

The viability of oocysts after 18 hours' incubation was tested by oral inoculation of one litter of newborn specific pathogen free mice for each of the 13 treatments. Each mouse received 0·1 ml of the mixture of rat gut homogenate and disinfectant and, after an incubation period of five days, one mouse per litter was killed on each of the following six days. Giemsa stained smears were made of the contents of the large bowel and portions of gut from upper, middle and lower small intestine were fixed in 10 per cent formol saline for histological examination. The presence of cryptosporidium oocysts in large bowel contents (shedding of oocysts) and, or, observation of organisms adhering to the brush borders of enterocytes in the small intestine were regarded as evidence of viability of the initial inoculum. Survival of oocysts after a 10 second incubation was taken as the base line to which counts taken after two hours' and 18 hours' incubation were related.

TABLE 1: Disinfectants recommended* for use in veterinary investigation laboratories

Disinfectant	Recommended concentration
Cresylic acid	3 per cent
Hypochlorite solution	2 to 5 per cent
Formaldehyde	10 per cent
Benzylkonium chloride	5 per cent
Ammonia†	5 to 10 per cent
Sodium hydroxide†	0·02M
Iodophore†	1 to 4 per cent

* Ministry of Agriculture, Fisheries and Food (1978)
† Used routinely in the laboratory as well as the recommended disinfectants

Results

Table 2 shows that, with the exception of formol saline, an incubation period of two hours in disinfectant had little or no effect on the numbers of oocysts. After 18 hours' incubation, 0·33 per cent iodophore, formol saline and 5 per cent cresylic acid reduced the oocyst numbers by more than five-fold. In all three treatments most of the oocysts observed after 18 hours appeared morphologically to be damaged. The normally smoother circular outline (Fig 1) was irregular and oocysts were shrunken and intensely stained (Figs 2a and b).

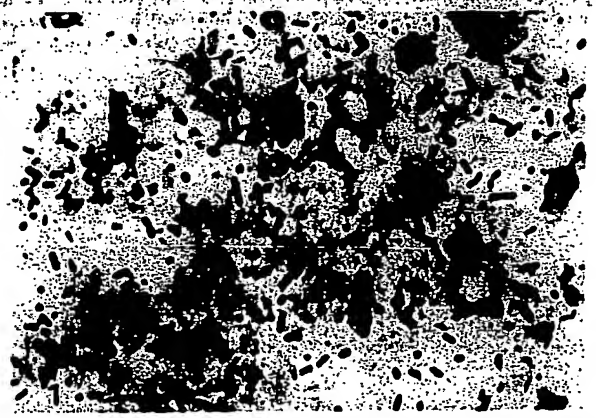

FIG 1: Normal cryptosporidium oocysts under oil immersion × 1086

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TABLE 2: Survival of calf cryptosporidium oocysts after 18 treatments

Treatment of gut homogenate with	Oocyst count* after incubation of			Inoculation of six to eight mice after 18 hours' treatment†	
	10 seconds	2 hours	18 hours	Shedding of oocysts	Histological evidence
Distilled water	29	21	12	+	–
Phosphate buffered saline	28	21	22	+	+
0·33 per cent iodophore	28	21	5	+	+
1 per cent iodophore	32	20	13	+	+
4 per cent iodophore	31	10	9	+	–
10 per cent formol saline	22	1	20‡	–	–
2·5 per cent cresylic acid	29	22	5	–	+
5 per cent cresylic acid	13	5	9§	+	+
3 per cent sodium hypochlorite	32	26	20	+	+
5 per cent benzylkonium chloride	34	22	10	+	+
10 per cent benzylkonium chloride	9	22	20	+	–
5 per cent ammonia	29	22	11	–	–
0·02M sodium hydroxide	21	18	19	+	+
Uninoculated control mice				–	–

* Number of oocysts counted on Giemsa stained smear in two minutes
† Gut contents and histological sections examined between five and 11 days after inoculation
‡ 20 oocysts seen, 16 appeared morphologically damaged
§ All oocysts appeared morphologically damaged

The viability tests in specific pathogen free mice indicated that only ammonia and formol saline completely destroyed oocyst infectivity (Table 2).

Discussion

These results show that cryptosporidium oocysts, like those of other coccidia (Levine 1973) are extremely resistant to common disinfectants. This organism was also shown to be resistant to the actions of a variety of antimicrobial agents (Tzipori and others 1982).

Unlike coccidia, cryptosporidia generally produce only limited numbers of small (4 µm) oocysts which are often difficult to detect in animals that are effected only subclinically. So far it has not been found possible to purify or concentrate cryptosporidial oocysts by methods used for other enteric coccidia (eg, flotation or sporulation techniques). Thus the test system used was not sufficiently sensitive for the results to be quantified, because evidence of infection in mice, which remain clinically healthy, is based on oocyst shedding and, or, histological demonstration of attached organisms. The two methods were therefore regarded as complementary.

With some of the disinfectants, infection, and therefore viability of oocysts, was demonstrated either by oocyst shedding (10 per cent benzylkonium chloride) or attachment to enterocytes (2·5 per cent cresylic acid) but not both.

If the oocysts had been less resistant the results would have been more difficult to interpret. This is because failure to demonstrate infection in mice could have been interpreted either as susceptibility to the disinfectant used, or the insensitivity of the test system. By methods used it was clearly demonstrated that only formol saline and ammonia, both powerful disinfectants, completely destroyed oocyst viability.

The infective material used in the test was tissue homogenate and therefore appreciable quantities of protein must have been present. This could have influenced the activity of some of the disinfectants. This problem will only be resolved when separation of oocysts from the gut content or faeces can be achieved.

The damaged oocysts observed after 18 hours' incubation with formol saline suggest that the morphological (and perhaps antigenic) characteristics of some of the oocysts were retained despite the loss of viability which could have occurred earlier. Further (unpublished) data suggest that cryptosporidium oocysts are also susceptible to damage by freezing at −70°C and freeze-drying with or without the incorporation of bovine albumin or glycerol as cryoprotectants.

Because cryptosporidium lacks host specificity and is therefore potentially zoonotic (Tzipori and others 1980a), and because the survival time of the oocyst under natural conditions is unknown, these findings could have important implications in control measures against contamination in laboratories or laboratory animal premises. In the light of our results, fumigation with formaldehyde or ammonia would be the most appropriate form of decontamination.

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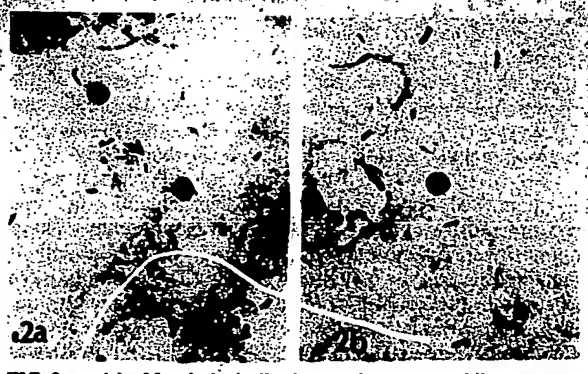

FIG 2a and b: Morphologically damaged cryptosporidium oocysts after 18 hours' treatment with 5 per cent cresylic acid ×1086

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